

DETERMINATION OF ANTIMICROBIAL ACTIVITY OF FRUITS

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ABSTRACT

*The present study was designated to study the antimicrobial activities of aqueous extracts, that were prepared from fresh fruits of *Embolica officinalis*, *Hylocereus undatus*, *Vitis vinifera*, *Syzygium cumini* and *Actinidia deliciosa*. The antimicrobial activities of the selected fruit extracts were evaluated, by measuring the zones of inhibition using Agar well Diffusion method, against five species of micro organisms; *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. The overall result of this study indicated that, the fruits showed different inhibition zones, along with different concentrations and observed that, the effect of fruits against pathogens were concentration dependent. Response against in increase and decrease in concentration was varied, among all the fruits. Fruits might have antimicrobial activity, against different Gram-positive, Gram-negative and yeast pathogens and could be used for prevention of various diseases, caused by these organisms.*

KEYWORDS: Fruits, Antimicrobial & Diseases

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INTRODUCTION

Nowadays, antibacterial therapy is mainly focussed on the administration of antibiotics and synthetic drugs. The indiscriminate use of antibacterial, particularly the antibiotics, has resulted in the emergence of resistant microbial strains and accumulation of metabolites in tissues and fluids, finally culminating in toxicity and adverse side effects (Noor *et al.*, 2004). The increased demand for effective and safer therapeutics has resulted in the renewed interest for use of natural products, in improving health and fitness. Medicinal plants are rich sources of bioactive compounds, such as alkaloids, flavonoids and phenolic compounds (Kannan *et al.*, 2009). Fruits are amongst the first food items, known to human beings. Fruits, whether fresh or dried, have always formed a part of the staple diet of human beings (Nayak *et al.*, 2012). The reason for this was that, they were rich in nutrients and provide some of the essential minerals, vitamins, to our body. Apart from that, they also help in curing a number of diseases.

Embolica officinalis (commonly known as Nelli, Amla, Amalaki or Indian gooseberry), is a small and medium sized deciduous tree found throughout India, Sri Lanka and Malaysia, the fruits of which are highly valued in traditional medicine (Khan, 2009). *H. undatus*, or commonly known as white pitaya, owing to its white flesh *H. undatus* originate from southern part of Mexico and it is now widely introduced in Asian countries, such as Taiwan, Malaysia and Vietnam, as well as northern Australia (Lim *et al.*, 2012). Grape (*Vitis vinifera* L.) is one of the most important commercial fruit crops of temperate, to tropical regions (Gowda *et al.*, 2008). Grape belongs to the Vitaceae family (Jansen *et al.*, 2006), native to the Mediterranean region, central Europe and southwest Asia and cultivated today in all temperature regions of the world (Gruenwald *et al.*, 2004).

Jamun (*Syzygium cumini*) commonly known as, Indian blackberry and belongs to the Myrtaceae family.

It is widely distributed throughout India, Ceylon-Malaya and Australia. Jambul, java plum, Indian blackberry and black plum are common names of jamun (Ali *et al.*, 2013) and has been valued in Ayurveda and Unani system of medication, for possessing variety of therapeutic properties (Kirtikar and Basu, 1975). Kiwi fruit belongs to the genus *Actinidia* (Actinidiaceae) and is derived from a deciduous woody, fruiting vine. Kiwi plants were originally grown in mountainous, forested regions of China (as Chinese gooseberry) (Ferguson and Ferguson 2003 and Motohashi *et al.*, 2002). *A. deliciosa* fruit has translucent, green flesh with rows of edible, black seeds, covered by a brown and hairy skin.

Many pharmacological studies have demonstrated the antioxidants, antidiabetic, antilipase, anticarcinogenic and anti-inflammatory activities on fruits viz, Amla, Dragon Grape Jamun & Kiwi. However, studies have not focused much on the antimicrobial effects of fruits, eaten ripe or unripe. The present study aims at screening for antimicrobials, in the raw ripe fruit. Water extracts are used for their close resemblance, to the traditional way of the fruit consumption. Water extracts of the ripe fruit parts are screened for antimicrobial properties.

MATERIALS AND METHODS

Preparation of Fruit Extract

Fruits viz., Amla (*Emblia officinalis*), Grape (*Vitis vinifera*) and Jamun (*Syzygium cumini*) were procured from growers located in and around Chennai. Imported fruits viz., Dragon (*Hylocereus undatus*) and Kiwi (*Actinidia deliciosa*) were procured from super market. The fruits were thoroughly rinsed in tap water and then distilled water, separately. The whole fruits were pureed well using a sterilized juicer, and then filtered through a muslin cloth to obtain a clear aqueous fraction of fruit, free from pulp (Waterhouse *et al.*, 2009). The juice was then kept in tight container, and was stored in -20° C, before further analysis.

Bacterial Cultures

Bacteria viz., *Pseudomonas aeruginosa* (MTCC2453), *Klebsiella pneumonia* (MTCC109), *Escherichia coli*, (MTCC1303) *Staphylococcus aureus* (MTCC96) and fungus like *Candida albicans* (MTCC 227) were procured from the Microbial type culture collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The pure bacterial cultures were maintained on nutrient agar medium, and fungal culture on potato dextrose agar medium. Each bacterial and fungal culture was further maintained by sub culturing regularly, on the same medium and stored at 4°C, until use in experiments.

Agar Well Diffusion Method

Antimicrobial activity of different fruit extracts were evaluated by Agar Well Diffusion method (Murray *et al.*, 1995), modified by Olurinola (1996). Nutrient agar and Potato dextrose agar plates were swabbed (sterile cotton swabs) with 8 hour old broth culture of respective bacteria and fungi. Wells (10mm diameter and about 2cm a part) were made in each of these plates, using sterile cork borer. Different volume (50µl, 75µ and 100µl) of fruit extracts were added, using sterile syringe into wells and allowed to diffuse at room temperature for 2 hours. Chloramphenicol was used as a standard antibiotic in 10mg ml⁻¹ range. The plates were incubated at 37° C for 18-24 h, for bacterial pathogen and 28° C hours, for fungal pathogens. The diameter of the inhibition zone (mm) was measured, and activity was calculated.

$$\text{Inhibition (\%)} = \frac{\text{Dia. of zone of Control (mm)} - \text{Dia. of zone of fruit extract (mm)}}{\text{Diameter of zone of Control (mm)}} \times 100$$

Statistical Analysis

The data obtained were analyzed statistically, using the Software of Statistical Package for Social Sciences (SPSS 16.0) and as per the standard procedure adopted, by Snedecor and Cochran (1994).

RESULTS AND DISCUSSIONS

Tables 1 to 5, show the mean \pm SE values for antimicrobial activity of aqueous extracts of amla, dragon, grape, jamun and kiwi fruits, against *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. From the data presented in the tables 1 to 5, it was found that, the antimicrobial activities of different concentrations in fruit samples ($P < 0.01$), differ highly significant. The zone of inhibition of the isolates, was found to be a function of the relative antibacterial potency of the extracts. Thus, zones of inhibition decreased as the concentration of the extracts decreased (Figure 1-5).

From table 1, aqueous extract of amla (100 μ l), exhibited the maximum activity against *Klebsiella pneumonia* (16.50 \pm 0.342mm), followed by *Escherichia coli* (14.33 \pm 0.33mm), *Staphylococcus aureus* (14.33 \pm 0.42mm), *Pseudomonas aeruginosa* (13.33 \pm 0.33mm) and *Candida albicans* (10.33 \pm 0.21mm). The bactericidal activity of amla, could be attributed to the bioactive compounds present in amla viz., flavonoids, phenols, saponins, and tannins (emblicanin A and B), which could be effectively employed as effective chemotherapeutic agents, in antibacterial treatment and therapy (Jyothi and Subba, 2011 & Javale and Sabnis, 2010).

Table 1: Evaluation of Antimicrobial Activity in Aqueous Extract of Amla (Mean \pm SE)[@]

Name of the Sample	Vol. (μ l)	Zone of Inhibition (mm)				
		<i>Pseudomonas Aeruginosa</i>	<i>Klebsiella Pneumonia</i>	<i>Escherichia Coli</i>	<i>Staphylococcus Aureus</i>	<i>Candida Albicans</i>
Amla	50	11.33 \pm 0.42 ^a	14.50 \pm 0.50 ^a	11.17 \pm 0.40 ^a	12.00 \pm 0.44 ^a	9.50 \pm 0.22 ^a
	75	11.67 \pm 0.42 ^a	14.67 \pm 0.33 ^a	13.50 \pm 0.34 ^b	13.33 \pm 0.42 ^b	9.50 \pm 0.22 ^a
	100	13.33 \pm 0.33 ^b	16.50 \pm 0.34 ^b	14.33 \pm 0.33 ^b	14.33 \pm 0.42 ^b	10.33 \pm 0.21 ^b
F value		84.174**	25.551**	62.511**	16.049**	28.118**

@- Average of six trials

** - Highly Significant ($P \leq 0.01$)

Different superscripts in a same column differ significantly $P \leq 0.01$

From table 2, the maximum antimicrobial activity was observed in aqueous extract (100 μ l) of dragon fruit, against *P. aeruginosa* (12.67 \pm 0.21mm), followed by *E. coli* (12.67 \pm 0.21mm), *K. pneumonia* (10.67 \pm 0.21mm), *S. aureus* (10.50 \pm 0.22mm) and *C. albicans* (10.67 \pm 0.21mm). The results obtained were in agreement with the results of Nurmahani *et al.* (2012), who studied the aqueous extract of dragon fruit, and found to exhibit highest antibacterial activity against the pathogenic and food spoilage micro organisms viz., *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. The antimicrobial activity was mainly, due to the presence of bioactive compounds present in the dragon fruit.

Table 2: Evaluation of Antimicrobial Activity in Aqueous Extract of Dragon Fruit (Mean± SE)[@]

Name of the Sample	Vol. (μl)	Zone of Inhibition (mm)				
		<i>Pseudomonas Aeruginosa</i>	<i>Klebsiella Pneumonia</i>	<i>Escherichia Coli</i>	<i>Staphylococcus Aureus</i>	<i>Candida Albicans</i>
Dragon	50	10.33±0.21 ^a	10.17±0.16 ^a	10.17±0.16 ^a	10.00±0.00 ^a	9.50±0.22 ^a
	75	11.67±0.33 ^b	10.17±0.16 ^a	11.33±0.42 ^b	10.50±0.22 ^b	9.67±0.21 ^a
	100	12.67±0.21 ^c	10.67±0.21 ^a	12.67±0.21 ^c	10.50±0.22 ^b	10.67±0.21 ^b
F value		170.538**	90.000**	138.395**	323.333**	21.970**

[@]- Average of six trials

**- Highly Significant (P ≤ 0.01)

Different superscripts in a same column differ significantly P ≤ 0.01

From table 3, the maximum antimicrobial activity was found in aqueous extract of grape (100 μl), against *S. aureus* (16.00±0.36mm), followed by, *K. pneumonia* (13.67±0.21mm), *E. coli* (13.67±0.21 mm), *P. aeruginosa* (13.33±0.49 mm) and *C. albicans* (10.33±0.21mm). The present study was found complementary to the report of Baydar *et al.* (2004) and Ozkan *et al.* (2004), in which they showed that, different mixture of solvents containing polyphenols from black grape, significantly inhibited the growth of *S. aureus*, *K. pneumonia*, *P. aeruginosa* and *C. albicans*.

Table 3: Evaluation of Antimicrobial Activity in Aqueous Extract of Grape (Mean± SE)[@]

Name of the Sample	Vol. (μl)	Zone of Inhibition (mm)				
		<i>Pseudomonas Aeruginosa</i>	<i>Klebsiella Pneumonia</i>	<i>Escherichia Coli</i>	<i>Staphylococcus Aureus</i>	<i>Candida Albicans</i>
Grape	50	12.00±0.25 ^a	11.67±0.33 ^a	10.33±0.33 ^a	12.33±0.49 ^a	9.83±0.16 ^a
	75	12.83±0.40 ^{ab}	12.67±0.33 ^b	13.50±0.34 ^b	14.17±0.30 ^b	10.17±0.30 ^{ab}
	100	13.33±0.49 ^b	13.67±0.21 ^c	13.67±0.21 ^b	16.00±0.36 ^c	10.33±0.21 ^{ab}
F value		77.111**	30.283**	149.795**	50.098**	2.281 ^{NS}

[@]- Average of six trials

**- Highly Significant (P ≤ 0.01)

NS-Non significant

Different superscripts in a same column differ significantly P ≤ 0.01

From table 4, the maximum antimicrobial activity was observed in aqueous extract (100 μl) of jamun, against *S. aureus* (16.67±0.21mm), followed by *K. pneumonia* (16.00±0.44 mm), *E. coli* (15.33±0.33 mm), *P. aeruginosa* (14.50±0.22 mm) and *C. albicans* (10.17±0.30mm). The result of the present study was in line with a study observed by Priya *et al.* (2013), in which antimicrobial activity of jamun fruit extract, against *S. aureus*, *E. coli* and *C. albicans* were determined the selected microorganisms, showing potent antimicrobial activity.

Table 4: Evaluation of Antimicrobial Activity in Aqueous Extract of Jamun (Mean± SE)[@]

Name of the Sample	Vol. (μl)	Zone of Inhibition (mm)				
		<i>Pseudomonas Aeruginosa</i>	<i>Klebsiella Pneumonia</i>	<i>Escherichia Coli</i>	<i>Staphylococcus Aureus</i>	<i>Candida Albicans</i>
Jamun	50	11.67±0.21 ^a	11.67±0.21 ^a	12.17±0.16 ^a	14.67±0.33 ^a	10.33±0.21 ^a
	75	13.67±0.21 ^b	14.50±0.34 ^b	14.33±0.21 ^b	16.33±0.33 ^b	10.00±0.25 ^a
	100	14.50±0.22 ^c	16.00±0.44 ^c	15.33±0.33 ^c	16.67±0.21 ^b	10.17±0.30 ^a
F value		169.296**	58.333**	104.737**	28.194**	5.741**

[@]- Average of six trials

**- Highly Significant (P ≤ 0.01)

Different superscripts in a same column differ significantly P ≤ 0.01

Table 5: Evaluation of Antimicrobial Activity in Aqueous Extract of kiwi (Mean± SE)[@]

Name of the Sample	Vol. (μl)	Zone of Inhibition (mm)				
		<i>Pseudomonas Aeruginosa</i>	<i>Klebsiella Pneumonia</i>	<i>Escherichia Coli</i>	<i>Staphylococcus Aureus</i>	<i>Candida Albicans</i>
Kiwi	50	10.50±0.22 ^a	12.00±0.25 ^a	10.67±0.33 ^a	11.17±0.16 ^a	9.67±0.21 ^a
	75	11.50±0.22 ^b	13.00±0.00 ^b	11.17±0.16 ^a	12.33±0.21 ^b	10.17±0.30 ^{ab}
	100	12.33±0.33 ^b	14.83±0.16 ^c	12.17±0.16 ^b	14.17±0.30 ^c	10.33±0.21 ^{ab}
F value		204.831**	161.538**	225.286**	214.444**	4.386 ^{NS}

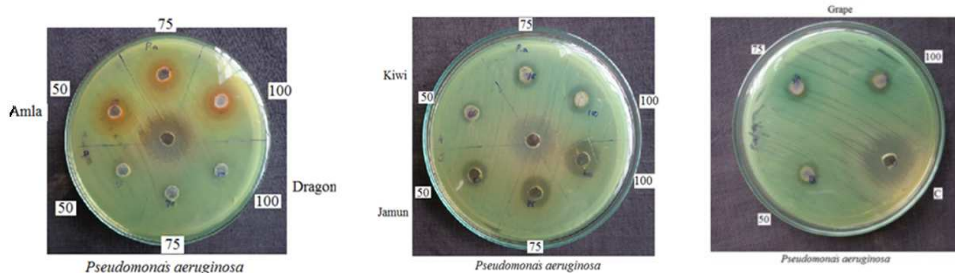
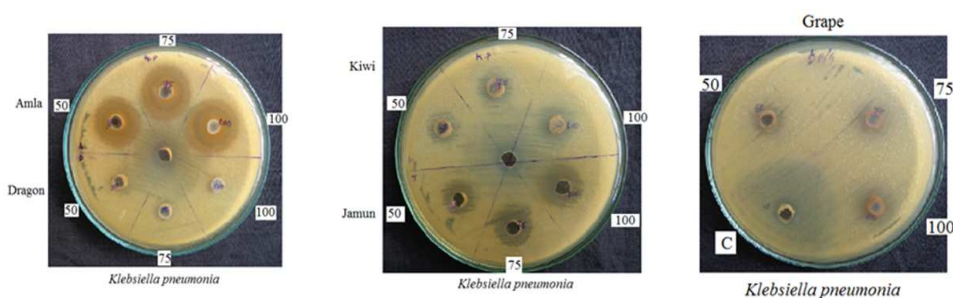
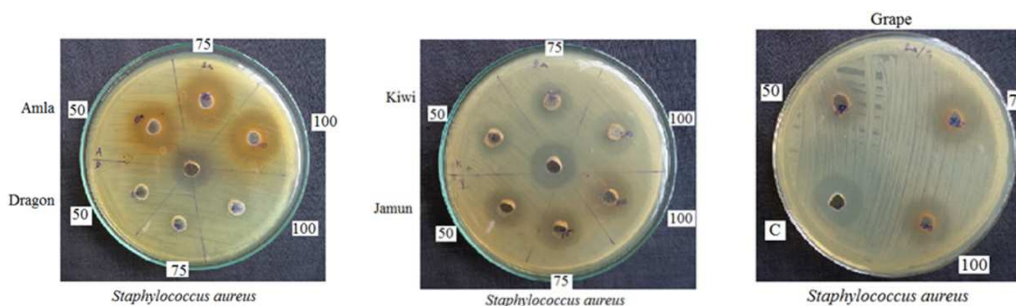
[@]- Average of six trials

** - Highly Significant ($P \leq 0.01$)

NS-Non significant,

Different superscripts in a same column differ significantly $P \leq 0.01$

From table 5, the maximum antimicrobial activity was found in aqueous extract (100 μl) of kiwi against *K. pneumonia* (14.83±0.16mm) followed by, *S. aureus* (14.17±0.30 mm), *P. aeruginosa* (12.33±0.33 mm), *E. coli* (12.17±0.16 mm) and *C. albicans* (10.33±0.21mm). Nessma Ahmed (2015) concluded that, kiwi extracts showed the highest antibacterial effects against *S. aureus*, *K. pneumonia* and *C. albicans*. The results of the present study were in concurrence with the findings recorded by, Mishra *et al.* (2010), in which the maximum inhibition was shown by methanolic and aqueous extract of kiwi fruit, against *E. coli*.

**Figure 1: Antimicrobial Activity of Fruits against Pseudomonas Aeruginosa****Figure 2: Antimicrobial Activities of Fruits against Klebsiella Pneumonia****Figure 3: Antimicrobial Activity of Fruits against Staphylococcus Aureus**

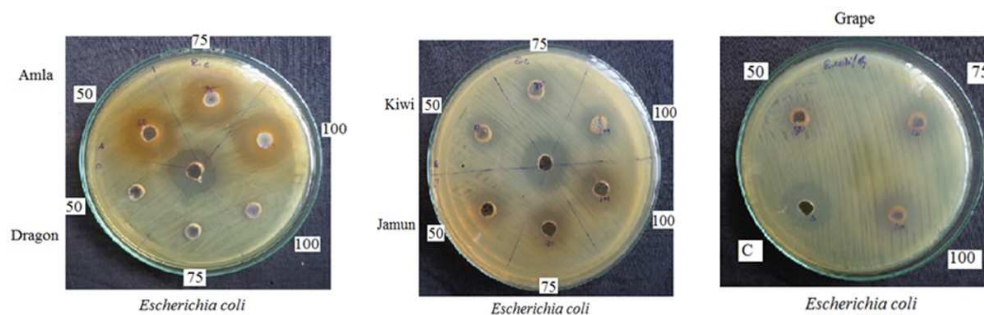


Figure 4: Antimicrobial Activities of Fruits against Escherichia coli

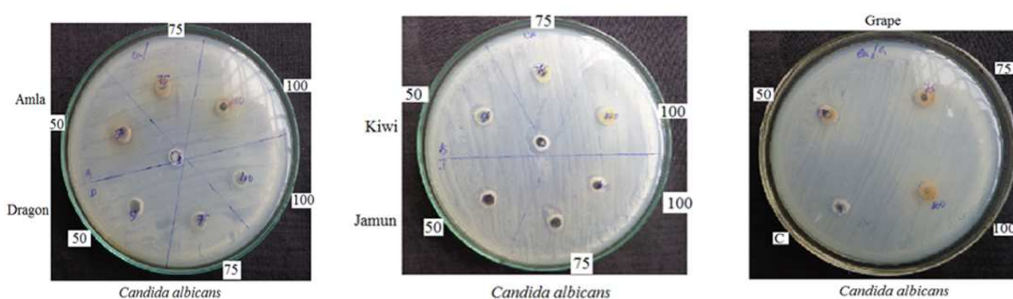


Figure 5: Antimicrobial Activities of Fruits against Candida Albicans

CONCLUSIONS

The results of this study have revealed that, these commonly consumed fruits may contain promising antimicrobial activities. This study, however, provides in vitro data, which may not be exactly replicated in vivo. Further studies directed at isolation of novel antimicrobial compounds and in vivo studies, which may validate the in vitro findings are recommended.

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